As described above, for the stabilization of phenobarbital, it is necessary that phenobarbital be dissolved in acidic solution, that is, in its molecular form, and not in its ionic from which is decomposed rapidly. The solubility of phenobarbital, however, is far below that for practical use, especially on the acidic side. Water-soluble organic substances, such as propyleneglycol, acetamide, diethyline (glycerin diethyl ether), are commonly added for its solubilization. When a small amount of water is mixed in these preparations, then phenobarbital crystallizes out. In order to overcome these difficulties, it is desirable to prepare a stable and water-soluble complex compound of phenobarbital for its stabilization.

The authors wish to express their thanks and appreciation to Prof. Dr. H. Nogami of University of Tokyo, for his guidance and encouragement throughout this study, and also to Dr. T. Okamoto and Mr. M. Horioka for their discussions.

Summary

The degradative reaction of phenobarbital was investigated over a pH range of 6.0 to 10.5 from the standpoint of chemical kinetics and the following results were obtained.

1) The degradation is an apparent unimolecular reaction at any fixed pH value and is in itself a bimoleculer reaction catalyzed by hydroxyl ion. The presence of any catalysis besides that by hydroxyl ion is not recognized. The relation between $\log k$ and pH is given in Fig. 4.

2) The results obtained well agreed with Eq. (8) which is derived from the postulation described in the theoretical consideration. As seen in Table III, the velocity constant k_1 (molecular form) is larger than k_2 (ionized form), but the decomposition depends on the concentration of hydroxyl ion, and hydrolysis in an alkaline region is very much accelerated.

3) The velocity constant k_2 was slightly smaller than that calculated and further investigation may be necessary for the elucidation of this reason.

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10. Shoji Shibata and Mikio Yamazaki : The Biogenesis of Plant Products. I. The Biogenesis of Rutin.

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The biological aromatization mechanism has chiefly been investigated by Davis,[1] Sprinson,[2] Tatum,[3] and their co-workers using microörganisms, and the participation of 5-dehydroquinic, 5-dehydroshikimic, and shikimic acids in the biosyntheses of phenyl, *p*-hydroxyphenyl, and 3,4-dihydroxyphenyl derivatives has extensively been elucidated. As for higher plant products, the incorporation of shikimic acid has only been shown by Eberhardt[4] in the lignin formation in sugar cane plant.

An entirely different biogenetical route of aromatization has been suggested for

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1) B.D. Davis : "Amino Acid Metabolism," Johns Hopkins Press, Baltimore, 799(1955).
2) P.R. Srinivasan, D.V. Sprinson, E.B. Kalan, B.D, Davis : J. Biol. Chem., **223**, 913(1956), and earlier papers.
3) E.L. Tatum, S.R. Gross : *Ibid.*, **219**, 797(1956).
4) G. Eberhardt, W.J. Schubert : J. Am. Chem. Soc., **78**, 2835(1956).

phloroglucinol and resorcinol formation.

Collie[5] proposed in 1907 a hypothesis of head-to-tail linkage of acetic acid unit to form various phenolic natural products. This idea has recently been revived[6~8] and some experimental evidences have been provided by Birch and his co-workers.[9]

It is assumed that a flavonoid would quite usefully be adopted for studies on biological aromatization in higher plant, since it frequently comes out with both phloroglucinol and catechol rings in its structure.

A biogenesis of cyanidine derivative (rubrobrassicin) in red colewort was reported by Weigand, Brucker, Griesebach, and Schulze,[10] and by Griesebach,[11] while our present work was in progress, and the incorporation of acetic acid(*methyl*-^{14}C) and (*carboxyl*-^{14}C) in phloroglucinol ring of cyanidine with head-to-tail linkage has been established.

Fagopyrum cymosum MEISN.[12] was used as the plant material for the present investigation of biosynthesis of rutin which was isolated from the leaves in a fairly good yield.

Acetate(*methyl*-^{14}C) was administered to the aerial part of the plant which was cultivated hydroponically for 6 days, and rutin isolated was degraded into phloroglucinol and catechol portions to measure their radioactivity.

Experimental

Cultural Condition—*Fagopyrum cymosum* MEISN. wildly growing in the campus of this University was lifted from the ground on June 20, 1957, after sunset. The aerial part was removed from roots, and cultivated hydroponically from the next day for 6 days in the following medium (the Houghland solution) : KNO_3, 5×10^{-3}; $Ca(NO_3)_2\cdot4H_2O$, 5×10^{-3}; $MgSO_4\cdot7H_2O$, 2×10^{-3}; KH_2PO_4, $1\times$

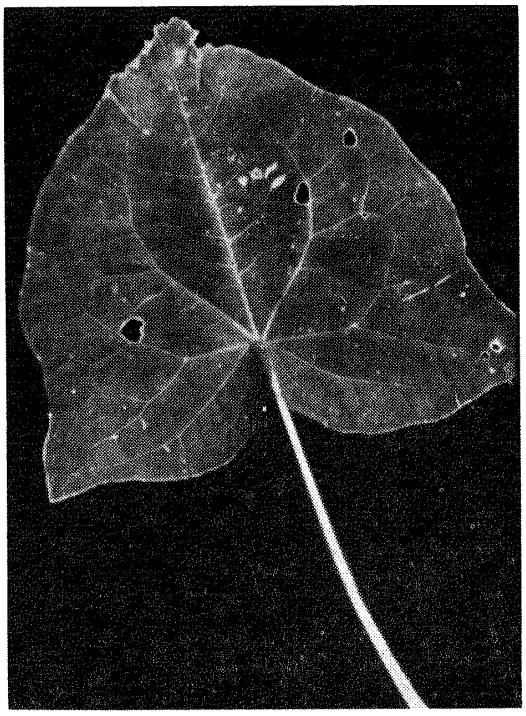


Fig. 1.

5) J.N. Collie : J. Chem. Soc., **63**, 329(1879); *ibid.*, **91**, 1806(1907).
6) R. Robinson : "The Structural Relations of Natural Products," The Clarendon Press, Oxford (1955).
7) A.J. Birch, F.W. Donovan : Austral. J. Chem., **6**, 360(1953).
8) A.J. Birch : "Perspective in Organic Chemistry," 147(1956).
9) A.J. Birch, R.A. Massy-Westropp, R.W. Rickards, H. Smith : Proc. Chem. Soc., **1957**, 98.
10) F. Weigand, W. Bruckers, H. Griesebach, E. Schulze : Z. Naturforsch., **12**, b., 222(1957).
11) H. Griesebach : *Ibid.*, **12**, b., 227(1957).
12) K. Imai, K. Furuya : Yakugaku Zasshi, **71**, 266(1951).

10^{-3}, $^{14}CH_3COONa$, 3×10^{-1}(0.125 mc.); minor elements, trace. (The figures indicate mol./L.)

The plant (500 g.) was divided into two bottles, each of which was filled with 900 cc. of the medium; the culture was carried out in a closed system as indicated in the previous paper.[13] The temperature range recorded during the cultivation was 25~30°. The radio-autogram of the leaves after 6 days' cultivation is shown in Fig. 1.

Extraction—The fresh leaves (200 g.) were removed from the stem after 6 days' cultivation and extracted 4 times with MeOH. The methanolic extract was concentrated *in vacuo* to separate chlorophyll. Rutin crystals were finally obtained from the concentrated extract and were recrystallized from aq. EtOH to yellowish needles, m.p. 186°. Yield : 900 mg.

Degradation of Rutin—Rutin (800 mg.) was subjected to hydrolysis by heating with 0.5% H_2SO_4 (80 cc.) to give quercetin, glucose, and rhamnose. Quercetin was recrystallized from aq. EtOH (yield, 380 mg.), and glucose and rhamnose were separated as their phenylosazones, recrystallized from 40%$(Me)_2CO$. D-Glucophenylosazone, m.p. 207°; L-rhamnophenylosazone, m.p. 186°.

Quercetin (380 mg.) was treated with CH_2N_2 in MeOH for 48 hrs. to obtain quercetin pentamethyl ether, m.p. 148°. Yield, 120 mg. Quercetin pentamethyl ether (60 mg.) was refluxed for 8 hrs. in 20% ethanolic KOH (10 cc.). Phloroglucinol monomethyl ether, m.p. 80°, was obtained as the phenolic portion, by recrystallization from benzene, and veratric acid, m.p. 178°, was isolated, which was recrystallized from hot water (yield, 26 mg.).

Veratric acid (25 mg.) was subjected to Schmidt reaction with conc. H_2SO_4 (0.3 cc.) and NaN_3 (20 mg.) in a small amount of benzene. The apparatus used for the Schmidt reaction was given in a previous paper.[14] Carbon dioxide evolved was trapped in $Ba(OH)_2$ solution to form $BaCO_3$ (14 mg.). The amine fraction was not isolated in a crystalline state.

Results and Consideration

The degradation of rutin was carried out as shown in Chart 1 and the radioactivity of each degradation products was measured by "Q"-gas flow counter (Nuclear Instrument and Chemicals Corp.) attached to the scaler (Kaken, Model 32). The specific activities were calculated at the infinite thinness level. The radioactivity of rutin showed that it forms in the aerial part of the plant.

The participation of radioactive acetate was evidently shown by this experiment to be higher in phloroglucinol portion than in catechol ring of rutin molecule.

Table I. The Incorporation of Acetate(*methyl*-^{14}C) indicated by the Radioactivity of the Degradation Products of Quercetin

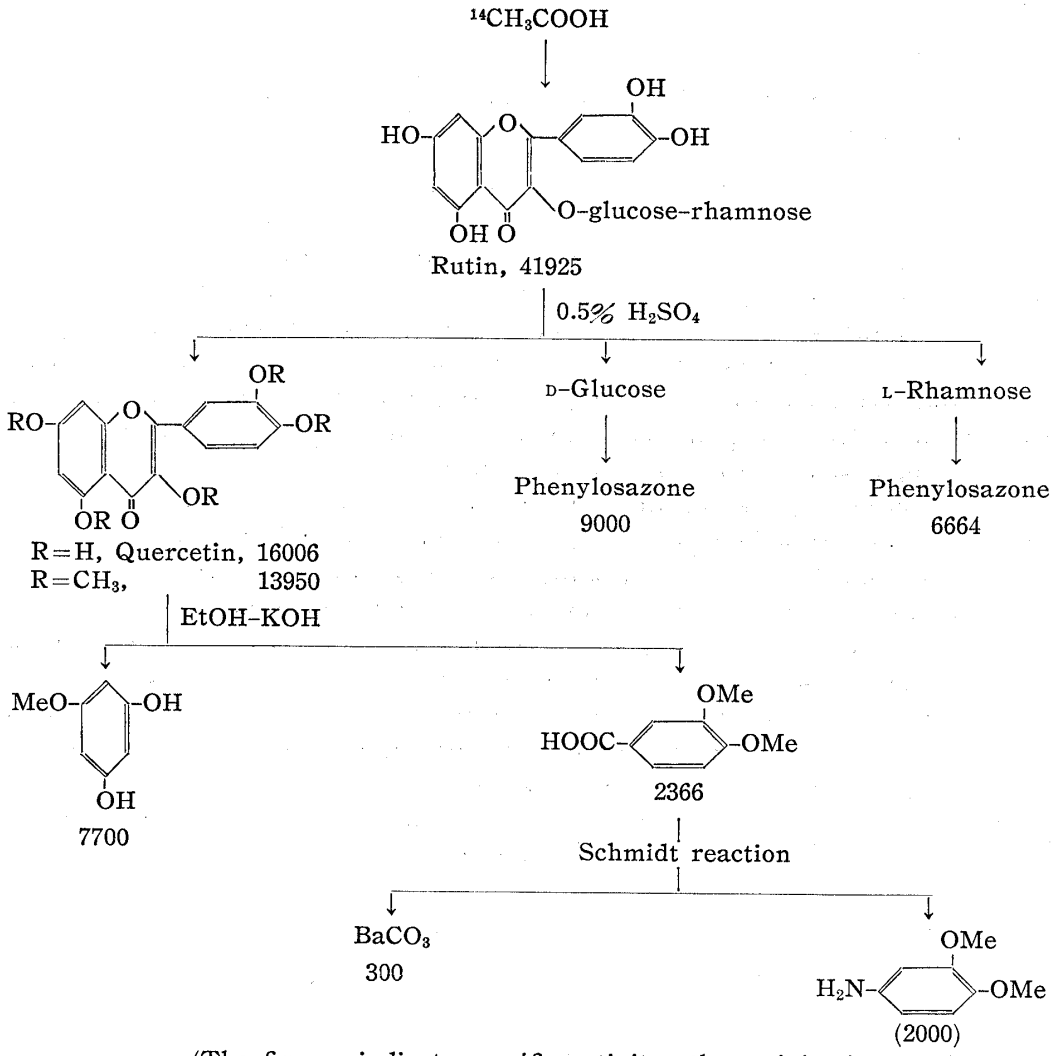
Compound	Specific Activity (c.p.m./mM.)	Incorporation based on the activity of quercetin (%)
Quercetin	13950	100
Phloroglucinol monomethyl ether	7700	56
Veratric acid	2366	16

Similarly to the result given by Griesebach with anthocyanin in red colewort,[11] the present study also gave an evidence that phloroglucinol ring of flavone was directly biosynthesized by head-to-tail linkage of the acetate unit.

The obvious difference of participation of acetate as observed in the radioactivities of phloroglucinol and catechol portions of rutin (or quercetin)(Table I) suggested that the latter would come indirectly by a different route of biosynthesis, probably via shikimic acid, though precise evidence has not yet been provided.

(Added later in manuscript) After the present work has been completed, we learned in the latest arrived journal that Neish, *et al.*[15] and Geissman, *et al.*[16] reported the biosynthesis of quercetin in buckwheat using ^{14}C-labeled acetate, and they gave the same conclusion as was obtained by us.

13) S. Shibata, I. Imaseki, M. Yamazaki : This Bulletin, **5**, 71(1957).
14) S. Shibata, I. Imaseki, M. Yamazaki : This Bulletin, **5**, 601(1957).
15) E.W. Underhill, J.E. Watkin, A.C. Neish : Canad. J. Biochem. Biophys., **35**, 219(1957).
16) T.A. Geissman, T. Swain : Chem. & Ind. (London), **1957**, 984.



(The figures indicate specific activity; the activity in parentheses was calculated by reduction)

Chart 1.

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Summary

The aerial part of *Fagopyrum cymosum* MEISN. was hydroponically cultivated for 6 days in the Houghland solution added with radioactive $^{14}CH_3COONa$. Rutin isolated from the leaves was degraded into phloroglucinol and catechol portions, and the specific activity of the degradation fragments was determined. The result indicated that the acetate was directly and dominantly incorporated into phloroglucinol ring in the rutin molecule and an evidence for the head-to-tail linkage of acetate in the biosynthesis of phloroglucinol was provided.

The catechol ring of rutin was suggested to be derived indirectly by a different route of biosynthesis.

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